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ORIGINAL PAPER

Pre-sowing treatment for breaking dormancy in *Acer velutinum* Boiss. seed lots

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Abstract: *Acer velutinum* Boiss is a valuable tree species native to Iran, and its seeds possess physiological dormancy that hampers seedling production in the nursery for large-scale reforestation efforts. The aim of this study was to determine the optimal dormancy breaking treatments for *A. velutinum* seeds. We conducted a factorial experiment involving six seed lots collected along an elevation gradient from 300 to 1800 m at 300 m interval and four cold-moist stratification periods (0, 4, 8 and 16 weeks) at 4°C and 70% relative humidity. The result shows that the germination of cold-moist stratified seeds was significantly ($p < 0.0001$) higher than the control for all seed lots. The highest germination capacity was recorded after 16 weeks of cold-moist stratification for all seed lots (68%–88% depending on the seed lot) except those collected from mid altitude sites (600 and 900 m) that germinated equally well (≥ 75%) after 4- and 8-week of clod-moist stratification compared to the other seed lots. The mean germination time was significantly shorter (12 to 19 days, depending on the seed lot) for seeds stratified for 16 weeks than for untreated seeds. It can be concluded that: (1) cold-moist stratification for 16 weeks is the best pre-sowing treatment for breaking dormancy in *A. velutinum* seeds; and (2) seeds should be collected from mid altitude sites (600 and 900 m) to get more than 80% germination within 15 days, and these seed lots even required shorter cold-moist stratification period (eight weeks) than other seed lots.

Keywords: cold-stratification; altitude; physiological dormancy; velvet maple; seed germination

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Introduction

The genus *Acer* (commonly known as Maple) is comprised of 150 species that are widely distributed in the temperate zone, ranging from North America, Europe, and Asia to North Africa. Maples are highly valuable for a variety of purposes, such as lumber and veneer, ornamental values, food and shelter for wildlife and watershed protection. *A. velutinum* Boiss (velvet maple) is one of the most valuable native species in Iran, which is widely distributed in Alborz Mountain from 200–2,000 m. Under favorable conditions, velvet maple reaches over 40 m in height and exceeds more than 150 cm in diameter. It comprises nearly 8% of the growing stocks in this area and provides nearly 2% of domestic wood demands (Farhadi et al. 2007). Its fast growing ability has drawn the attention of forest managers as one of the key species in reforestation of deforested or degraded lands. However, the dormancy of the seeds has hampered attempts to raise seedlings in the nursery for large-scale planting (Difazio et al. 1988; Yousef-Zadeh et al. 2007).

Seed dormancy is one of the adaptation mechanisms to promote plant survival by dispersing germination in space and time until environmental conditions are conducive for germination (Bewley and Black 1994; Baskin and Baskin 2001). Basically, seed dormancy is an innate seed property that defines the environmental condition in which the seed is able to germinate and produce a normal seedling. *Acer* seeds possess varying degree of dormancy and germinability (Baskin and Baskin 2001; Phartyal et al. 2003a), ranging from the non-dormant seeds of spring-fruiting species (*A. saccharinum*) through several intermediate forms to the deeply dormant seeds of fall-fruiting species (*A. Tataricum, A. caesium*). Dormancy in *Acer* seeds is attributed to covering structure (testa) that restricts imbibition and/or factors

in the embryo (Pinfield and Dungey 1985; Phartyal et al. 2003b). Thus, dormant *Acer* seeds should be pre-treated before sowing to get good germination and desirable quantity of seedlings in the nursery.

Dormancy in many seeds, including *Acer* species, can be released by low temperature, light and hormonal treatments (*e.g.* Phartyal et al. 2003a; Soltani et al. 2005; Sivakumar et al. 2006; Tigabu et al. 2007). In traditional nursery practice, cold stratification on moist medium for a certain length of time or autumn-sowing is often used to break dormancy in *Acer* seeds. The optimal duration of cold – moist stratification, however, varies between species and seed lots within the same species (Baskin and Baskin 2001; Phartyal et al. 2003a). For instance, Phartyal et al, (2003a) have shown that *A. caesium* seeds from the Mandal site required shorter stratification period than the Mussoorie seed lot, indicating source variation in the degree of dormancy. This accentuates the need for developing species-specific stratification protocol. Although progresses have been made in developing methods for breaking seed dormancy in *Acer* species elsewhere, little is known about *A. velutinum* in its natural habitat in Iran, except a study on the variation in seed germination in relation to seed source (e.g. Yousef-Zadeh et al., 2007). Therefore, there is still a great need to develop dormancy breaking method for *A. velutinum* seeds.

Thus, the main aim of our study was to find the best way to alleviate the dormancy of *A. velutinum* seeds, and the specific research questions addressed were: (1) What is the optimal cold stratification period for breaking the dormancy of *A. velutinum* seeds?; (2) Does the seed source have an influence on degree of dormancy and germinability of *A. velutinum* seeds? (3) Does the effect of stratification treatment vary with seed sources? To answer these questions, we collected seeds from six sites in Shast-Kalateh forest of Iran that vary in elevation from 300 to 1,800 m, and exposed seeds to cold-moist stratification for four, eight and 16 weeks at 4 °C and 70% relative humidity. The stratified and untreated seeds were then germinated and their overall and speed of germination were compared.

Materials and Methods

Seed collection

Seeds of *A. velutinum* were collected from Shast-Kalateh forest located at 36°42' N and 54°21' E with approximately 1,714 ha in the southwestern range of Gorgan city in the Caspian Forests located in Golestan Province (Fig. 1). The area is characterized by mean annual precipitation and temperature of 650 mm and 17.74°C, respectively and characterized as a semi-humid temperate area (Anonymous 2001). Seed lots were collected from 10 phenotypically superior trees at six sites differing in elevation, i.e. 300, 600, 900, 1,200, 1,500 and 1,800 m and covering the natural distributional range of the species. The seeds were manually extracted and cleaned, and sound seeds (without any visible damage) were used in the experiment.

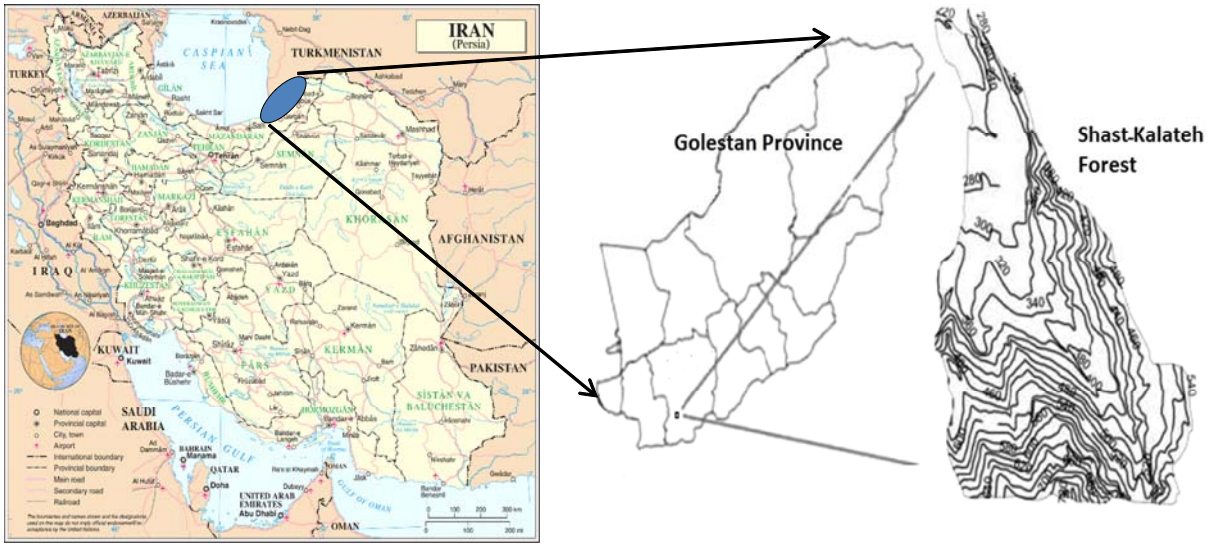

Fig. 1 Location of Shast-Kalateh forests, Northern Iran from where *A. velutinum* seeds were collected for the present study (Source: Etminan *et al.* 2011).

Cold-moist stratification

A 6 × 4 factorial experiment was designed to test the effects of seed source and the length of cold-moist stratification period on breaking dormancy in *A. velutinum* seeds. Seeds collected from each altitudinal site were stratified in petri dishes (9 cm) sandwiched between two germination papers that were kept moist with 2 ml deionized water for 4, 8 and 16 weeks at 4°C and 70% relative humidity. It has been shown that prolonged cold stratification up to 24–48 weeks at 5°C is effective to overcome the

dormancy of some *Acer* seeds (Phartyal et al. 2003a), thus we tested 16 weeks of stratification in this study. To prevent the papers from drying, 1-mL deionized water was added every two days.

Germination test

Directly after cold-moist stratification, germination tests were carried out under laboratory conditions at a constant temperature of 20±1 °C day and night with continuous illumination of 20 µE (Fluorescent lamp F 40 M/33 RS cool white light) for 48 days to allow sufficient time for the germination process to be completed. For each treatment, four replicates of 25 seeds per petri-dish were sown on moist germination paper in disinfected petri dishes. Untreated seeds were also sown as control. The germination of seeds was monitored every day, and the seeds were considered germinated when the radicle reached 5 mm long with a normal appearance.

Data analysis

For all germination tests, germination capacity (GC) and mean germination time (MGT) were calculated as:

$$GC(\%) = (\frac{\sum n_i}{N}) \times 100$$

$$MGT(days) = \frac{\sum t_i \times n_i}{\sum n_i}$$

where n_i is the number of seeds that germinated at each day, N is the total number of seeds sown, and t_i is the number of days starting from the date of sowing (Bewley and Black 1994). Prior to data analyses, all percentage data sets were arcsine transformed to meet the normality assumption for ANOVA (Zar 1996). General Linear Model (GLM) – Univariate Analysis was performed to determine significant differences in germination capacity and mean germination time among seed lots and cold-moist stratification periods. When interaction effects were detected, One-Way ANOVA was performed for each seed lot separately. Means that exhibited significant differences were compared by Tukey's Honestly Significant Test at the 5% level of significance. All data analyses were performed with Minitab 16.0 statistical software (Minitab Inc., State College, PA, USA).

Results

The release of dormancy in *A. velutinum* seeds, as shown by germination capacity and mean germination time, varied significantly with respect to cold-moist stratification periods, seed lots and their interaction (Table 1). Generally, cold-moist stratification treatments resulted in significantly higher germination than the control for all seed lots (Fig. 2). The highest germination capacity was recorded after 16 weeks of cold-moist stratification for all seed lots except those collected from mid altitude sites (600 and 900 m). Seed lots from the mid altitude sites germinated equally well (≥ 75%) after 4- and 8-week of clod-moist stratification compared to the other seed lots. Even the untreated seeds germinated in the range of 30% (seed lot collected from 1,500 m site) to 48% (seed lot collected from 900 m site).

Table 1. Summary of GLM-Univariate analysis for testing significant effects of seed sources and cold-moist stratification periods on the germination capacity (GC) and mean germination time (MGT) of *A. velutinum* seeds.

Variable	Source of variation	d.f. *	Adj. MS*	F-value	P-value
GC	Seed source (SS)	5	0.235	72.19	< 0.0001
	Stratification period (SP)	3	1.20	368.57	< 0.0001
	SS × SP	15	0.029	8.97	< 0.0001
	Error	72	0.003		
MGT	Seed source (SL)	5	139.64	24.32	< 0.0001
	Stratification period (SP)	3	1262.10	219.82	< 0.0001
	SS × SP	15	12.22	2.13	< 0.0001
	Error	72	5.74		

d.f. = degrees of freedom; Adj. MS = Adjusted Mean square

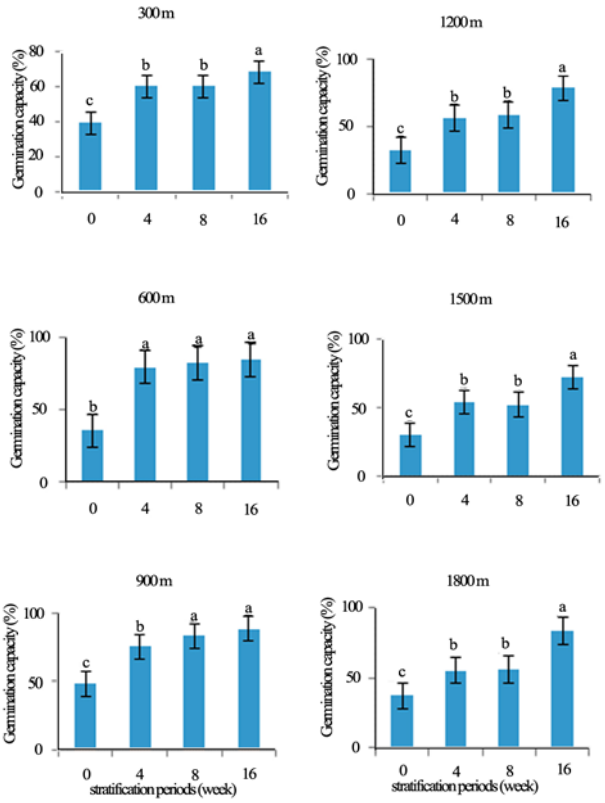

Fig. 2 Germination capacity (%) **of *A. velutinum* seed lots collected from six elevations in response to different cold-moist** stratification **periods (Mean ± SE)**. Bars with different letter are significantly different using Tukey's Honestly Significant test (α = 0.05)

The cumulative germination curves showed a distinct difference in the speed of germination between stratified and untreated seeds (Fig. 3). Seeds stratified for 16 weeks started to germinate during the first week of the germination trial, and germination increased sharply within 21 days after sowing as compared to the sluggish germination of untreated seeds. Seeds stratified for eight weeks also showed a similar pattern as that of 16-week stratified seeds, particularly for seed lot collected from 900 m site. The overall mean germination time of seeds was significantly shorter for stratified than untreated seeds; particularly cold-moist stratification for 16 weeks significantly reduced the average time to germinate for all seed lots compared to other stratification treatments (Table 2). Among seed lots, seeds collected from 600, 900 and 1800 m sites germinated quickly once the dormancy was broken by cold-moist stratification for 16 weeks. Cold-moist stratification for eight weeks also resulted in quicker germination of seeds collected from 900 m site than seeds collected from other sites.

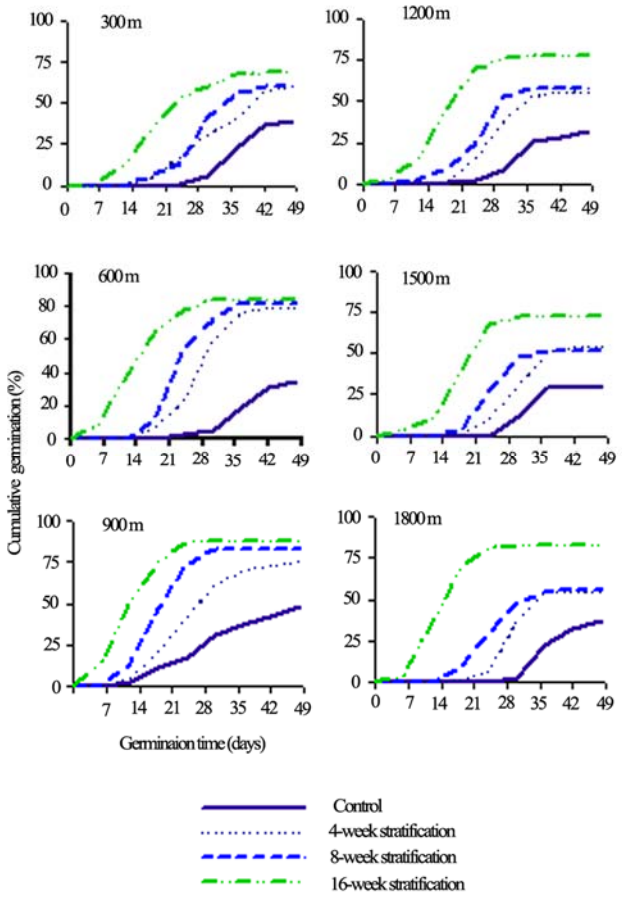

Fig. 3 The speed of germination of *A. velutinum* seed lots collected from six elevations in response to different cold-moist stratification periods.

Table 2. Mean germination time (days) of *A. velutinum* seed lots collected from six elevations in response to different cold-moist stratification periods (Mean ± SE)

Seed source	Cold-moist stratification period (weeks)			
(elevation m a.s.l)	0	4	8	16
300	35.8 ± 1.1**A**	30.6 ± 2.7**AB**	27.8 ± 1.6**B**	19.4 ± 0.5**C**
600	35.4 ± 1.2**A**	26.4 ± 0.9**B**	24.1 ± 0.8**B**	14.4 ± 0.2**C**
900	25.0 ± 0.9**A**	23.2 ± 2.1**A**	17.7 ± 0.5**B**	12.0 ± 0.3**C**
1200	32.9 ± 2.0**A**	28.0 ± 0.5**B**	23.9 ± 0.7**B**	17.2 ± 0.7**C**
1500	31.4 ± 1.1**A**	29.5 ± 1.4**A**	24.8 ± 0.5**B**	17.3 ± 1.2**C**
1800	36.7 ± 0.4**A**	28.6 ± 0.5**B**	23.8 ± 1.8**C**	13.8 ± 0.9**D**

Means followed by the same letter across the rows are not significantly different using Tukey's test ($\alpha = 0.05$).

Discussion

Dormancy in *A. velutinum* seeds is caused by factors in the embryo (physiological inhibitory mechanisms) rather than the covering structure (testa) as evidenced from some germination of untreated seeds (30%–48% depending on the seed lot) and high germination performance of stratified seeds (Fig. 2). Seeds with physiological dormancy need certain environmental cues that trigger metabolic changes culminating in germination (Bewley and Black 1994; Baskin and Baskin 2001). For many tree seeds exhibiting physiological or morpho-physiological seed dormancy, cold stratification at 1–10°C (in some cases as high as 15°C) increases germination (Teketay 1997; Baskin and Baskin 2001; Phartyal et al. 2003a; Soltani *et al.* 2005; Tigabu et al. 2007).

Exposure to low temperature (cold-stratification) triggers a cascade of physiological processes, involving changes in protein expression and synthesis, energy and methionine metabolism as well as transcription and signal transduction (Krawiarz and Szczotka 2000; Pawlowski, 2010). Shen and Odén (2002) showed that fumarase activity (a key respiratory enzyme in the tricarboxylic acid cycle) in stratified seeds of European beech was twice higher than in dormant seeds. Low temperature treatment also influences the balance of germination promoting (GA) and germination inhibiting (ABA) phytohormones. For oriental beech, eight weeks of cold-stratification reduced the ABA level in the embryonic axis by 15-fold compared to the control, and an inverse relationship was observed between ABA level and germination (Soltani, 2003). Cold-stratification increases the sensitivity of seeds to GA, perhaps through elevating bioactive GAs by repressing the GA 2-oxidase gene family (Yamauchi *et al.* 2004). The expression of protein phosphatase 2C (FsPP2C1) and other genes that appears in dormant seeds and in ABA-treated seeds tends to decrease or disappear during cold stratification, which partly explains the decline of ABA content in the embryonic axis during cold stratification (Lorenzo et al. 2001).

The length of cold stratification period required for dormancy release largely depends on the extent of dormancy (Baskin and Baskin 2001) and varies among populations from different elevations (Milberg and Andersson 1997; Lohengrin and Arroyo 2000). The available evidence suggests that the stratification

period necessary for germination increases with elevation of seed source in plant species growing along altitudinal gradients (Dorne 1981; Tigabu et al. 2007). For example, cold stratification for one month was enough to trigger germination of *Phacelia* seeds collected from 1,600 m site whereas seeds collected from higher altitude (3,600 m) site required three months of clod stratification (Dorne 1981). As temperature decreases with increasing elevation, seed lots from higher elevation are naturally exposed to low temperature for a longer period, thus, they might have developed an adaptation mechanism to low temperature as dormancy breaking cue. In the present study, *A. velutinum* seeds collected from mid altitude sites had higher germination capacity than seeds collected from higher altitude sites after eight weeks of cold-stratification, which was also comparably high with that of 16-week cold stratification. Exception to this was the long cold-stratification period (16 weeks) needed by *A. velutinum* seeds collected from low altitude (300 m) site to yield 68% germination (Fig. 2). Although no dormancy breaking treatments were applied, Yousef-Zadeh et al. (2007) have also observed significant differences among seed lots from different elevations in germination and seedling survival of *A. velutinum* from the Mazandaran forests in Iran.

One possible explanation for this would be the quality of the seed lots. Seeds from this site might have low vigor; thus, needed more time to germinate. The quality of a given seed lot and its germinability can be markedly influenced by maternal factors, such as position of the seed in the fruit/tree, the age of the mother plant during seed maturation, as well as environmental factors such as day length, temperature, light quality, water availability and altitude (Wulff 1995; Mamo et al. 2006, Tigabu et al. 2007), as well as seed ageing that commences at physiological maturity and continues during harvest, processing and storage, which, in turn is related to genetic and environmental factors (McDonald 1999). Seed deterioration results in reduced overall germination performance, speed and uniformity of germination, inferior seedling emergence and growth. This can be further corroborated by the cumulative germination curve (Fig. 3) where the germination process was still progressing and had not reached the breaking point for some of the seed lots, notably seed lot collected from 300 m site. It is also legitimate to expect variations in age and vitality of mother trees, as *A. velutinum* seeds for this study were collected from natural stands. Interestingly, the untreated seeds of *A. velutinum* germinated to a varying extent and rate depending on the seed source (Fig. 2), suggesting variability in level of dormancy within a given seed lot. Although reproductive fitness in plants is generally governed by the genetic constitution (Bazzaz et al. 2000), maternal environmental effects during seed maturation cause variation in germinability of seeds developed on the same mother plant and on plants of the same species growing in different environments (Gutterman 2000).

Cold stratification for 16 weeks has shortened the mean germination time to about 15 days compared to more than 30 days for the control for most of the seed lots (Table 2). The increase in the speed of germination, thus, avoiding uneven and erratic emergence over long periods, is a goal much desirable by nursery workers. Maple growers in the USA, for example, prefer to achieve 80% germination in two weeks following sowing of cold-stratified seeds (Young and Young 1992), which may perhaps be the same wish elsewhere. Rapid germination also offers another advantage in that it lessens the risk to plant establishment due to fungal attacks while in the seedbed. In addition, at the end of the nursery period seedlings of more homogenous size will generally be obtained.

Conclusions

The effects of different lengths of cold-moist stratification period at 4°C and 70% relative humidity on the release of dormancy in *A. velutinum* seed lots that were collected across an elevation gradient were investigated in the present study. Based on the findings, the following conclusions are made, which in turn improve seedling production in the nursery for large-scale plantations,

(1) Cold-moist stratification for 16 weeks is the best pre-germination treatment for breaking dormancy in *A. velutinum* seeds, as this treatment resulted in higher overall and speed of germination.

(2) Seed lots differ in their germinability following stratification treatments, and this might be attributed to their initial quality. In terms of overall and speed of germination, seed lots collected from mid altitude sites (600 and 900 m) are of superior quality.

(3) The length of cold-moist stratification period varies with the seed lots. Seed lots that originated from mid altitude sites (600 m and 900 m) had the highest germination and even require shorter cold-moist stratification period (eight weeks) than other seed lots. Thus, these seed lots should be used in future collection if natural stands will continue to be the source of seed for the nurseries

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